

A Canadian Bison Isolate of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) Is Not Transmissible by *Dermacentor andersoni* (Acari: Ixodidae), Whereas Ticks from Two Canadian *D. andersoni* Populations Are Competent Vectors of a U.S. Strain

GLEN A. SCOLES,¹ TERRY F. McELWAIN,² FRED R. RURANGIRWA,² DONALD P. KNOWLES,
AND TIMOTHY J. LYSYK³

USDA-ARS, Animal Disease Research Unit, Washington State University, Pullman, WA 99164

J. Med. Entomol. 43(5): 971–975 (2006)

ABSTRACT *Anaplasma marginale* Theiler is a tick-borne rickettsial pathogen of cattle with a global distribution in both temperate and tropical regions. The pathogen is endemic in regions within the United States, whereas the Canadian cattle population is considered to be free of *A. marginale*. Farmed bison, *Bison bison* L., in central Saskatchewan have been found to be infected with *A. marginale*; however, there is no evidence of transmission from bison to cattle. We tested a Saskatchewan bison isolate of *A. marginale* (SB1) to determine whether it is transmissible by the Rocky Mountain wood tick, *Dermacentor andersoni* Stiles. Colonized *D. andersoni* from the United States and Canada failed to transmit SB1. A separate transmission trial using *D. andersoni* adults reared from ticks collected in Alberta and British Columbia showed that ticks from these populations could successfully transmit the St. Maries, Idaho, strain of *A. marginale*. Although the Saskatchewan bison isolate of *A. marginale* seems not to be transmissible by *D. andersoni*, in the event of the introduction of a tick-transmissible strain, Canadian *D. andersoni* are likely to be competent vectors.

KEY WORDS transmission, ticks, bison, *Dermacentor andersoni*, *Anaplasma marginale*

Anaplasma marginale Theiler is a tick-borne intraerythrocytic rickettsial pathogen of cattle. Acute infection can result in clinical anaplasmosis, which may be characterized by anemia, weight loss, abortion, and in some cases, death. Most cattle that survive acute infection seem to be clinically healthy but remain persistently infected for life. Persistently infected cattle serve as reservoirs for infection of competent tick vectors. The Rocky Mountain wood tick, *Dermacentor andersoni* Stiles, is the predominant biological vector of *A. marginale* in the U.S. intermountain west, where *A. marginale* is endemic. Ticks in U.S. populations of *D. andersoni* transmit *A. marginale* efficiently, even at the very low levels of infection characteristic of persistently infected cattle (Eriks et al. 1993). This tick species is broadly distributed throughout the western United States and southwestern Canada (Wilkinson 1967, James et al. 2006). Canadian cattle are considered to be free of *A. marginale* infection (Power 2000, OIE 2004). In 2000, *A. marginale* infection was detected in two commercial bison, *Bison bison* L., herds

in central Saskatchewan; 20 infected bison were destroyed to eradicate the outbreak. No additional cases have been detected in Canadian bison or cattle since that time (OIE 2004), even though cattle are susceptible to infection with both U.S. (Oklahoma) (de la Fuente et al. 2003) and Canadian (Saskatchewan) (unpublished observation) bison isolates of *A. marginale*.

The origin of the *A. marginale* infection in these Canadian bison is unknown. Nor is it known why cattle in Canada are free of *A. marginale* infection, even though a known vector and the pathogen are both present. Rapid response and elimination of infected animals in past outbreaks may have contributed to maintenance of Canada's anaplasma free status. However, there may also be biological reasons why Canadian cattle remain anaplasma free. Anecdotal accounts have suggested that Canadian *D. andersoni* are less competent vectors of *A. marginale*, but there seems to be no published data in support of this conjecture. Recently, *D. andersoni* collected from a site in British Columbia have been shown to develop midgut infection with *A. marginale* when fed on a persistently infected calf (Scoles et al. 2005b). Although transmission was not attempted in that study, infection of the midgut is a critical first step leading to tick-borne biological transmission. The failure of the Saskatche-

¹ Corresponding author, e-mail: scoles@vetmed.wsu.edu.

² Program in Vector-Borne Disease, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164.

³ Agriculture and Agri-Foods Canada Research Centre, Lethbridge, Alberta, Canada T1J 4B1.

wan bison *A. marginale* strains to be naturally spread to cattle may reflect their inability to be transmitted by ticks, because nontick transmissible isolates of *A. marginale* have been identified (Smith et al. 1986, Wickwire et al. 1987, de la Fuente et al. 2001).

We have examined the transmissibility of *A. marginale* strains from Idaho and Saskatchewan by ticks from U.S. and Canadian *D. andersoni* populations. In the first experiment, repeated attempts were made to transmit an *A. marginale* isolate from a Saskatchewan bison by using male and female *D. andersoni* from U.S. and Canadian sources. A second experiment was conducted to determine whether ticks from two Canadian populations of *D. andersoni* were capable of transmitting the St. Maries, Idaho, strain of *A. marginale*, which is known to be transmissible by ticks from a U.S. *D. andersoni* population.

Materials and Methods

***A. marginale* Strains.** The Saskatchewan bison isolate of *A. marginale* (SB1) was obtained from an animal in a commercially raised bison herd in which the overall prevalence of infection was 26%. Fragments of the 16S rDNA, *groesl* and *msp5* genes of SB1 had 100% identity with the homologous gene fragments of the Florida strain of *A. marginale* (GenBank accession nos. AF309867, AF414865, and M93392, respectively), confirming the species identification of this isolate. However the *msp1a* repeat structure of SB1 was unlike any previously reported *A. marginale* strain, confirming that SB1 is a unique *A. marginale* strain (T.F.M., unpublished data). Approximately 160 ml of blood was collected from a persistently infected bison in Saskatchewan, shipped overnight on ice, and injected intravenously into splenectomized bovine calf c876bl. The calf developed a clinical *A. marginale* infection that was confirmed by polymerase chain reaction (PCR), a commercially available competitive enzyme-linked immunosorbent assay (cELISA) (VMRD Inc., Pullman, WA), and observation of Giemsa-stained blood smears. Acute infection reached a peak percentage of parasitized erythrocytes (PPE) of 1.9% on day 49 postinoculation and then dropped to <1.0% and remained low for the duration of this study. This calf was used as the acquisition host. Stabilates of this first passage SB1 isolate have been cryopreserved in liquid nitrogen and are available for further study.

The St. Maries strain of *A. marginale* used in this study originated from *D. andersoni* collected off of a naturally infected bull from St. Maries, ID (Eriks et al. 1994). The stabilate used to infect the acquisition host was produced from a splenectomized calf that was infected by these field-collected ticks (one tick passage). In several different studies, this *A. marginale* strain has been shown to be transmissible by colonized *D. andersoni* and also has been shown to be transmissible by *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini) (Eriks et al. 1993, Futse et al. 2003).

Rocky Mountain Wood Ticks. *D. andersoni* used for the SB1 transmission trials were from laboratory colonies. The Reynolds Creek (RC) tick colony origi-

nated from Owyhee Co. in southwestern Idaho and has been maintained by feeding on cattle for >20 generations at the University of Idaho Holm Research Center. The Chin Lake (CL) *D. andersoni* were first generation (F₁) offspring of ticks collected near Chin Lake in southern Alberta, reared on rabbits at the Agriculture Agri-Foods Canada (AAFC) Research Centre in Lethbridge.

The St. Maries transmission trial was conducted using ticks originally collected from two geographically isolated sites. British Columbia (BC) ticks were collected near Walker Lake, ≈20 km south of Kamloops, British Columbia (50° 33' N, 120° 15' W). Alberta (AB) ticks were collected at the same site near Chin Lake where the CL ticks originated, ≈45 km east of Lethbridge, Alberta (49° 36' N, 112° 11' W). Field-collected ticks from each site were reared for one generation on rabbits. Experiments were conducted with adult male ticks, which were the F₁ offspring of the field-collected adult ticks. Voucher specimens, both intact ticks preserved in alcohol, and tick genomic DNA frozen at -70°C, are available for further study.

Cattle. All cattle used in these experiments were cared for following procedures approved by the University of Idaho Institutional Animal Care and Use Committee. Before use all calves were confirmed to be free of *A. marginale* infection by using cELISA and a nested PCR assay (Scoles et al. 2005a,b). With the exception of the original splenectomized calf that was subinoculated with bison blood for initial amplification of SB1, spleen intact male Holstein calves were used for acquisition and transmission hosts.

Bison *Anaplasma* Transmission Trials. Splenectomized calf c876bl was infected by subinoculation with bison blood, as described above, on 29 January 2001. Male ticks were acquisition fed on c876bl on three different occasions between 20 March 2001 (acute) and 8 January 2002 (persistent), and then transmission fed on uninfected, spleen intact calves on seven different occasions between 3 April 2001 and 5 February 2002; also, nymphal ticks were fed on c876bl on 8 January 2002 and then after they molted to the adult stage, both male and female ticks were transmission fed on uninfected, spleen intact calves on 5 March 2002. The infection level of the acquisition calf was monitored on Giemsa-stained blood smears, PPE on the feeding dates is presented in Table 1. Acquisition feedings lasted 7 d; ticks were removed and held at 25°C and 98% RH for 7 d and then placed on hosts for transmission feeding for 7–10 d. Dates and durations of acquisition and transmission feedings and the PPE at the time of acquisition feeding are detailed in Table 1. For both acquisition and transmission feedings, ticks were confined under muslin and stockinet feeding patches attached with hip tag cement to the shaved sides of each calf. A sample (12–16 ticks) of the RC and CL ticks that were acquisition fed on 8 January 2002 were dissected after transmission feeding, and the salivary glands and guts were tested for *A. marginale* infection by nested PCR (Scoles et al. 2005b). After tick transmission feeding, calves were monitored for

Table 1. Attempted transmission of the Saskatchewan bison *A. marginale* isolate SB1 by *D. andersoni*

Tick group	Acquisition feeding		Infection status ^c	PPE (%)	Transmission feeding			Tick infection		
	Date ^a	No. fed ^b			Host	Date ^a	No. fed ^d	n ^e	Gut + ^f	Saliv + ^g
RC	20 Mar. 2001	89	Acute	1.06 ^h	c886bl	3 April 2001	49	NT	NT	NT
RC	2 Aug. 2001	150	Persistent	BD ⁱ	c907bl	17 Aug. 2001 ^j	40	NT	NT	NT
					c906bl	22 Aug. 2001	45	NT	NT	NT
					c924bl	23 Jan. 2002	25	12	0	0
RC	8 Jan. 2002	34	Persistent	0.15 ^k	c925bl		24	12	1	0
RC		94			c926bl		24	12	0	2
CL		26			c927bl	5 Feb. 2002	25	16	2	0
CL		25			c932bl	5 Mar. 2002	81 ^m	NT	NT	NT
RC		103 ^l								

NT, not tested.

^a Acquisition and transmission feedings begin on the specified date and are completed in 7 d unless otherwise noted.

^b Number of acquisition fed ticks recovered at the end of the feeding interval.

^c Stage of infection of the acquisition calf.

^d Number of transmission fed ticks recovered at the end of the feeding interval.

^e n is number dissected.

^f Number of PCR-positive guts.

^g Number of PCR-positive salivary glands

^h Value ranged from 0.6 to 1.6% over the 7-d feed.

ⁱ PPE below detection.

^j Ten-day feed.

^k Value ranged from 0.10 to 0.24% over the 7-d feed.

^l Acquisition fed as nymphs; transmission fed after molting to adult.

^m Both males and females put on for transmission; 43 females and 38 males were recovered.

infection by using Giemsa-stained blood smears, nested PCR, and cELISA (Scoles et al. 2005a,b).

St. Maries *A. marginale* Transmission with Canadian *D. andersoni*. Calf c1066bl was inoculated with 2 ml of frozen first passage stabilate of the St. Maries strain of *A. marginale* on 23 December 2004. The PPE first dropped below detection on stained blood films on 22 February 2005 (day 61 postinoculation).

During feeding, male ticks were confined in stockinet sleeves attached to the shaved side of the calf with hip tag cement. The ticks were acquisition fed on c1066bl for 7 d (BC ticks 29 March–5 April; AB ticks 6–13 April 2005). The infection level of the calf at the time of feeding was below detection on stained blood smears but was determined using quantitative PCR (Scoles et al. 2005b). After acquisition feeding, ticks were removed and held off the host for 7 d at 25°C and 98% RH, before transmission feeding for 7 d on uninfected calves c1076bl and c1075bl (BC 12–19 April; AB 20–27 April 2005, respectively). A sample of the AB ticks was dissected after acquisition feeding (without transmission feeding), and all of the transmission-fed ticks from both calves were dissected after the transmission feeding. Tick guts and salivary glands were dissected as described by Scoles et al. (2005a,b), and were tested for *A. marginale* infection by nested PCR. The level of infection was determined in a sample of the salivary glands by quantitative PCR. After tick transmission feeding, calves were monitored for infection using Gimsa-stained blood smears, by nested PCR, and by cELISA, as described previously.

DNA Preparation and PCR. DNA from tick tissues was prepared using a modified IsoQuick (Orca Research, Bothell, WA) protocol, and nested PCR for the *A. marginale* MSP5 gene was preformed as described previously (Scoles et al. 2005a,b). Quantitative PCR was preformed as described previously (Scoles et al.

2005b) on a sample of tick salivary glands after transmission feeding (four AB and four BC ticks) to determine the number of genome copies of *A. marginale* per salivary gland pair.

Statistical Analysis. Numbers of infected and uninfected individuals were compared between the BC and AB populations by using the G test of goodness-of-fit for single-classification frequency distributions, applying Williams' correction for sample sizes <200 (Sokal and Rohlf 1987). The number of *A. marginale* genome copies per salivary gland pair was compared between ticks from the BC and AB populations by using Student's *t*-test on log-transformed data.

Results

Bison *Anaplasma* Transmission Trials. The Canadian bison isolate of *A. marginale* was not transmitted to any of the eight different hosts on which ticks were transmission fed. Transmission attempts were made during acute infection as well as at two points during persistent infection by using RC colony ticks with both the intrastadial (adult male acquisition and transmission) and interstadial (nymphal acquisition, adult transmission) transmission models, and with CL ticks by using the intrastadial transmission model (Table 1). A small proportion of the ticks that were dissected after transmission feeding had PCR-positive guts (8.3–12.5%) or salivary glands (16.7%) (Table 1).

St. Maries *Anaplasma* Transmission with Canadian *D. andersoni*. Ticks from both Canadian populations successfully transmitted the St. Maries strain of *A. marginale* from the persistently infected acquisition host to an uninfected calf (Table 2). At the time of acquisition feeding, the level of infection was 6.38×10^4 genome copies per ml, well below the threshold for microscopic detection. The proportion of ticks with

Table 2. Transmission of the St. Maries, Idaho, strain of *A. marginale* by *D. andersoni* from two Canadian populations

Tick group	Acquisition fed ^a	n	Gut+ ^b	Saliv+ ^c	Transmission fed ^d	n	Gut+ ^b	Saliv+ ^c	Transmission host	PP	PPE (%)
BC	22	NT	NT	NT	22	21	17	17	c1076bl	21	4.7
AB	61	30	29	25	25	24	24	22	c1075bl	23	4.0

n, number dissected; PP, prepatent period, i.e. days from initiation of transmission feeding to first detection on Giemsa-stained blood smears; NT, not tested.

^a Number of ticks acquisition fed.

^b Number of dissected ticks with nested PCR-positive guts.

^c Number of dissected ticks with nested PCR-positive salivary glands.

^d Number of ticks transmission fed.

PCR-positive guts and salivary glands was high (Table 2); there was no significant difference between the proportions of ticks with salivary gland infections in the BC group versus the AB group (80.95 versus 91.67%, respectively; $G_{adj} = 1.034$, $P > 0.05$) or between the proportions of salivary gland infections of the AB pre- and post-transmission feeding groups (83.3 versus 91.67%, respectively; $G_{adj} = 0.793$, $P > 0.05$).

After transmission feeding, the average number of *A. marginale* genome copies per salivary gland pair was similar between the BC ticks (5.81 log, SD = 0.32, $n = 4$) and the AB ticks (5.03 log, SD = 1.01, $n = 4$) (t -test on log-transformed data; $t = 1.48$; $df = 6$; $P = 0.1893$). Transmission calves c1075bl and c1076bl followed a normal course of *A. marginale* infection, rising to peak PPEs of 4.0 and 4.7% within 31 and 34 d of tick attachment, respectively (Table 2).

Discussion

Ticks from the Canadian and U.S. populations of *D. andersoni* were not able to transmit the SB1 isolate of *A. marginale*, whereas ticks from two Canadian populations of *D. andersoni* efficiently transmitted St. Maries, a well characterized U.S. *A. marginale* strain. These data suggest that the difference in prevalence of *A. marginale* infection between Canadian and U.S. cattle populations might be accounted for by differences in the transmissibility of the *A. marginale* strains that are present, rather than by differences in the vector competence of ticks for known *D. andersoni*-transmissible strains.

Transmission of the SB1 strain failed at levels of infection that were 3 orders of magnitude greater than the levels at which the St. Maries strain was efficiently transmitted. The PPE of the SB1-infected calf averaged 1.06% during the acute phase acquisition feeding and 0.15% during the persistent phase acquisition feeding. This represents between 1.1×10^7 and 7.4×10^7 infected erythrocytes per ml of blood. Compare this to 1.6×10^4 to 3.2×10^4 infected red blood cells per ml in the St. Maries calf at the time of transmission (0.0002–0.0005 PPE; 6.38×10^4 genome copies per ml adjusted for two to four organisms per infected red blood cell; see Scoles et al. 2005a).

The Office International des Epizooties (OIE), the World Organization for Animal Health, considers Canada to be free of bovine anaplasmosis based on

testing of 15,473 sera screened with the complement fixation test in 2000 (Power 2000, OIE 2004) and 15,300 sera screened in 2003 by using the newer and more sensitive cELISA test (OIE 2004). No additional cases of *A. marginale* infection have been detected in Canadian cattle or bison since the infected bison that were discovered in 2000 were destroyed (OIE 2004). The Saskatchewan bison isolate of *A. marginale* tested in this study originated from the bison outbreak in 2000. Because *A. marginale* is endemic in U.S. cattle, this pathogen constitutes one barrier to free trade between the United States and Canada. U.S. cattle can be shipped to Canada only under a specialized import regime called the Restricted Feeder Program, which allows imports of feeder cattle directly to feedlots without testing. Breeding cattle can only be imported into Canada from the United States if serological tests confirm that they are *Anaplasma*-free; this testing requirement is considered a nontariff trade barrier. Considerable interest is being focused on rationalizing regulations for importation of breeder cattle from the United States to Canada. A better understanding of the vector capacity of Canadian ticks for *A. marginale* and of the transmissibility of Canadian isolates could help inform both U.S. and Canadian import/export policy.

The origin, distribution, and mode of transmission of the *A. marginale* that was found infecting Canadian bison is not known. The SB1 isolate seems not to be transmissible by ticks in either Canadian or U.S. populations of *D. andersoni*, even though gut and salivary gland infection was detected in a small number (Table 1). The distribution of *D. andersoni* probably does not extend north to central Saskatchewan where SB1 was collected; however, vector competence of this species for SB1 would be important in the event of the movement of infected bison into areas where *D. andersoni* occurs. The most likely natural vector in central and northern Saskatchewan might be the winter tick, *Dermacentor albipictus* (Packard). The vector competence of *D. albipictus* for SB1 has not been tested, but a Texas strain of *D. albipictus* has been shown to be a competent vector for the Virginia strain of *A. marginale* (Stiller et al. 1981, Stiller and Kuttler 1983). Whether the failure of U.S. or Canadian *D. andersoni* to transmit SB1 would be characteristic of other *A. marginale* strains that might be found in Canada, or is just characteristic of this strain in particular, is an open question. However, we know that nontick transmissibility is not a general characteristic of bison isolates

of *A. marginale*, because an isolate from Oklahoma bison has been shown to be transmissible by *Dermacentor variabilis* (Say) (Kocan et al. 2004).

Field-collected ticks from populations of *D. andersoni* do differ in midgut susceptibility to infection with *A. marginale*, a key component of vector competence (Scoles et al. 2005b), suggesting that there are differences among tick populations in vector competence. However, the average number of *A. marginale* genome copies per salivary gland pair for the Canadian ticks tested in these studies was within the range of values previously reported for U.S. ticks: Scoles et al. (2005a) reported 6.8×10^6 genome copies per salivary gland pair; at earlier times in the salivary gland, Lohr et al. (2002) estimated that there were from 7.66×10^4 to 1.65×10^6 genome copies per salivary gland pair; Eriks et al. (1993) reported levels in whole male ticks between 4.06 and 4.96 logs (1.1×10^4 to 9.1×10^4). These data suggest that both the U.S. tick colony (RC) on which all of these estimates were based, and the Canadian tick populations tested here are equally competent vectors.

These data suggest that the strain of *A. marginale* collected from a bison in Canada is not transmissible by *D. andersoni*. It seems that the SB1 strain of *A. marginale* would be unlikely to spread to cattle by *D. andersoni*-borne transmission if infected bison were moved into areas where *D. andersoni* occurs. However, these data also suggest that in the event of an introduction or outbreak of a *D. andersoni*-transmissible strain of *A. marginale*, ticks in Canadian *D. andersoni* populations would be competent vectors.

Acknowledgments

We thank Sara Davis, Ralph Horn (USDA-ARS), R. C. Lancaster, and C. Himsel-Rayner (Agriculture and Agri-Food Canada) for superior technical assistance. This work was supported by USDA-ARS-CRIS Grant 5348-32000-016-00D.

References Cited

- de la Fuente, J., J. C. Garcia-Garcia, E. F. Blouin, B. R. McEwen, D. Clawson, and K. M. Kocan. 2001. Major surface protein 1a effects tick infection and transmission of *Anaplasma marginale*. *Int. J. Parasitol.* 31: 1705-1714.
- de la Fuente, J., E. J. Golsteyn-Thomas, R. A. Van Den Bussche, R. G. Hamilton, E. E. Tanaka, S. E. Druhan, and K. M. Kocan. 2003. Characterization of *Anaplasma marginale* Isolated from North American Bison. *Appl. Environ. Microbiol.* 69: 5001-5005.
- Eriks, I. S., D. Stiller, W. L. Goff, M. Panton, S. M. Parish, T. F. McElwain, and G. H. Palmer. 1994. Molecular and biological characterization of a newly isolated *Anaplasma marginale* strain. *J. Vet. Diagn. Invest.* 6: 435-441.
- Eriks, I. S., D. Stiller, and G. H. Palmer. 1993. Impact of persistent *Anaplasma marginale* rickettsemia on tick infection and transmission. *J. Clin. Microbiol.* 31: 2091-2096.
- Futse, J. E., M. W. Ueti, D. P. Knowles, Jr., and G. H. Palmer. 2003. Transmission of *Anaplasma marginale* by *Boophilus microplus*: retention of vector competence in the absence of vector-pathogen interaction. *J. Clin. Microbiol.* 41: 3829-3834.
- James, A. M., J. E. Freier, J. E. Keirans, L. A. Durden, J. W. Mertins, and J. L. Schlater. 2006. Distribution, seasonality, and hosts of the Rocky Mountain wood tick in the United States. *J. Med. Entomol.* 43: 17-24.
- Kocan, K. M., J. de la Fuente, E. J. Golsteyn-Thomas, R. A. Van Den Bussche, R. G. Hamilton, E. E. Tanaka, and S. E. Druhan. 2004. Recent studies on the characterization of *Anaplasma marginale* isolated from North American bison. *Ann. NY Acad. Sci.* 1026: 114-117.
- Lohr, C. V., F. R. Rurangirwa, T. F. McElwain, D. Stiller, and G. H. Palmer. 2002. Specific expression of *Anaplasma marginale* major surface protein 2 salivary gland variants occurs in the midgut and is an early event during tick transmission. *Infect. Immunol.* 70: 114-120.
- [OIE] Office International des Epizooties. 2004. Handistatus II, Canada/Bovine anaplasmosis, Multiannual animal disease status. Office International des Epizooties, the World Organization for Animal Health, Paris, France. (http://www.oie.int/hs2/sit_pays_mald_plasp?c_pays=32&c_mald=31).
- Power, C. 2000. Canadian cattle herds free of brucellosis, bluetongue and anaplasmosis. *Canadian Animal Health Network Bulletin*. Fall 2000, Edition 5, ISSN 1481-7074: 10.
- Scoles, G. A., A. B. Broce, T. J. Lysyk, and G. H. Palmer. 2005a. Relative efficiency of Biological transmission of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) by *Dermacentor andersoni* Stiles (Acari: Ixodidae) compared to mechanical transmission by the stable fly, *Stomoxys calcitrans* (L.) (Diptera: Muscidae). *J. Med. Entomol.* 42: 668-675.
- Scoles, G. A., M. W. Ueti, and G. Palmer. 2005b. Variation among geographically separated populations of *Dermacentor andersoni* (Acari: Ixodidae) in midgut susceptibility to *Anaplasma marginale* (Rickettsiales: Anaplasmataceae). *J. Med. Entomol.* 42: 153-162.
- Sokal, R. R., and F. J. Rohlf. 1987. Introduction to biostatistics. W.H. Freeman and Company, New York.
- Smith, R. D., M. G. Levy, M. S. Kuhlenschmidt, J. H. Adams, D. L. Rzechula, T. A. Hardt, and K. M. Kocan. 1986. Isolate of *Anaplasma marginale* not transmitted by ticks. *Am. J. Vet. Res.* 47: 127-129.
- Stiller, D., and K. L. Kuttler. 1983. Experimental Transmission of *Anaplasma marginale* by Males of *Dermacentor albipictus* (Packard) and *Dermacentor occidentalis* Marx (Acari: Ixodidae), pp. 59-65. In *Proceedings of the 87th Annual Meeting of the United States Animal Health Association*, 16-21 October, Las Vegas, NV. U.S. Animal Health Association, Richmond, VA.
- Stiller, D., G. Leatch, and K. L. Kuttler. 1981. *Dermacentor albipictus* (Packard): an experimental vector of bovine anaplasmosis, pp. 65-73. In *Proceedings of the 85th Annual Meeting of the United States Animal Health Association*, 11-16 October, St. Louis, MO. U.S. Animal Health Association, Richmond, VA.
- Wickwire, K. B., K. M. Kocan, S. J. Barron, S. A. Ewing, R. D. Smith, and J. A. Hair. 1987. Infectivity of three *Anaplasma marginale* isolates for *Dermacentor andersoni*. *Am. J. Vet. Res.* 48: 96-99.
- Wilkinson, P. R. 1967. The distribution of *Dermacentor* ticks in Canada in relation to bioclimatic zones. *Can. J. Zool.* 45: 517-537.

Received 13 February 2006; accepted 27 June 2006.